3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

CHO SERUM-FREE MEDIUM
Without L-Glutamine

Product Code **C 1707**
Storage Temperature 2-8 °C

Synonym: CHO SF Medium

Product Description

CHO Serum-free Medium is a complex formulation optimized for use in recombinant protein expression and production in Chinese Hamster Ovary (CHO) cell systems. It was developed to support high cell densities and maintain high cell viability for extended growth periods to maximize recombinant protein expression.

The expression of recombinant proteins has increased in importance in both research and pharmaceutical manufacturing applications. CHO cells are one of the most frequently used systems for the expression of recombinant proteins that require post-translational modification to express biological function.

As more recombinant proteins are being employed as therapeutic agents, the methods used in their production are coming under increasing regulatory scrutiny. One of the areas of concern is the use of serum-supplemented media for the culture of cells for recombinant protein expression. By using CHO SF Medium, regulatory concerns associated with the use of serum have been reduced.

Intended Use

For R&D use only. Not for drug, household or other uses.

Components

The formulation includes inorganic salts, HEPES, sodium bicarbonate, essential and non-essential amino acids, vitamins, bovine serum albumin, human transferrin, fetal bovine fetuin (USA source), trace elements, phenol red, Pluronic® F-68, and other organic compounds. The total protein concentration of CHO SF Medium is 100 µg/ml.

The medium does not contain L-glutamine, antibiotics, and antimycotics. It also does not contain hypoxanthine or thymidine. It can be used with dihydrofolate reductase (dhfr) gene amplification and glutamine synthetase systems.

Preparation Instructions

This medium is supplied as a sterile 1X liquid. Aseptically add 20 ml of 200 mM L-glutamine (Product Code G 7513) to each liter of medium prior to use. Supplementation with a surfactant (such as Pluronic® F-68) is not required.

Storage/Stability

This medium is stable, when stored at 2-8 °C and protected from light, until the indicated expiration date on the label.

Procedure

Freezing and Thawing

CHO cells grown in CHO SF medium have been successfully frozen in liquid nitrogen and recovered. Cells must be in the mid-logarithmic phase of growth with greater than 90% viability.

1. Pellet cells by centrifugation for 5 minutes at 200 x g. Re-suspend at a concentration of 5 x 10^6 cells/ml in a 50:50 mixture of fresh CHO SF Medium and conditioned CHO SF Medium supplemented with DMSO at a final concentration of 7.5%.
2. Freeze cells in liquid nitrogen according standard procedures (1 °C decrease per minute).
3. Recover cells by rapidly thawing the vial in a 37 °C water bath.
4. Dilute cells 1:10 in fresh CHO SF Medium. Mix and centrifuge the cell suspension at 200 x g for 5 minutes.
5. Re-suspend the pellet in 1 ml CHO SF Medium. Add 9 ml of fresh CHO SF Medium.
6. Transfer suspension to a T-75 flask containing fresh CHO SF Medium at a final volume of 30 ml. Suspension culture can be transferred to appropriate spinner culture after 2-3 days.

Adaptation to CHO SF Medium

Minimal time is required to adapt CHO cells from serum-containing medium to CHO SF Medium. For good adaptation, it is critical that cell viability is at least 90% and the cells are in the mid-logarithmic growth phase. Cells grown in serum-containing medium should be inoculated at a viable cell density of 2 x 10^5 cells/ml in a 1:1 mixture of serum-containing medium and CHO SF medium. Allow cells to reach a density of 1 x 10^6 cells/ml. Subculture at an initial density of 2 x 10^5 cells/ml into medium containing increasing proportions of CHO SF Medium, first at 1:3 mix and then 1:7 mix (serum-containing medium: serum-free medium). Titration may be required at each subculture step to achieve a good single-cell suspension. Cells are considered adapted when the cell density reaches 1 x 10^6 cells/ml. This usually occurs within 7 days after inoculation. The time interval required for adaptation will vary by individual CHO clone. All cultures should be incubated at 37 °C in a humidified atmosphere at 5% CO_2.

Product Profile

Sigma's CHO Serum-free Medium (Product Code C 1707) was compared to CHO media from three competitors (A, B, C) for growth and productivity. For these studies, CHO cells were adapted to the test media prior to the start of the experiments. Cells were then inoculated at a density of 2 x 10^5 cells/ml and grown in CHO Serum-free Medium or one of the competitors' formulations. DME/F12 supplemented with 10% FBS was included as an additional control. **Figure 1** illustrates that Sigma's CHO Serum-free Medium consistently supported the highest cell density and viability. **Figure 2** shows that CHO Serum-free Medium ranks at the top of commercially available products for productivity.

References

1. Merten, O.W., Safety issues of animal products used in serum-free media. Dev. Biol. Stand., **99**,167-180 (1999).
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3. Kim, E.J. et al., Development of a serum-free medium for dihydrofolate reductase-deficient Chinese hamster ovary cells (DG44) using a statistical design: beneficial effect of weaning cells. *In Vitro* Cell Dev. Biol. Animal. **35(4),** 178-182 (1999).
4. Bjare, U., Serum-free culture. Pharmacol. Ther., **53(3),** 355-374 (1992).

Precautions and Disclaimer MSDS is available upon request or at **sigma-aldrich.com**. Pluronic is a registered trademark of BASF Corporation.

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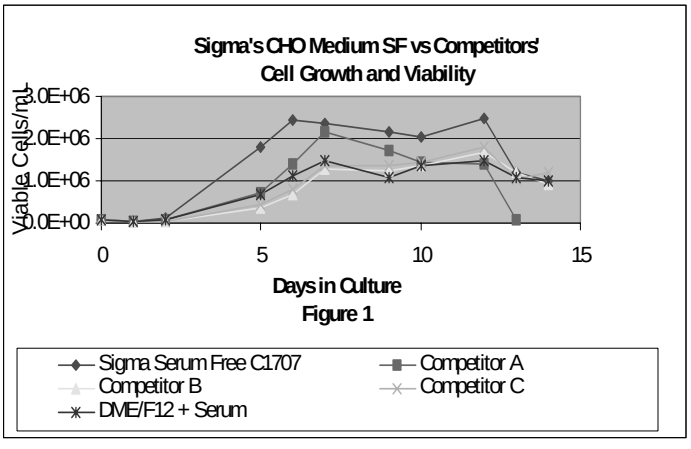

Figure 1

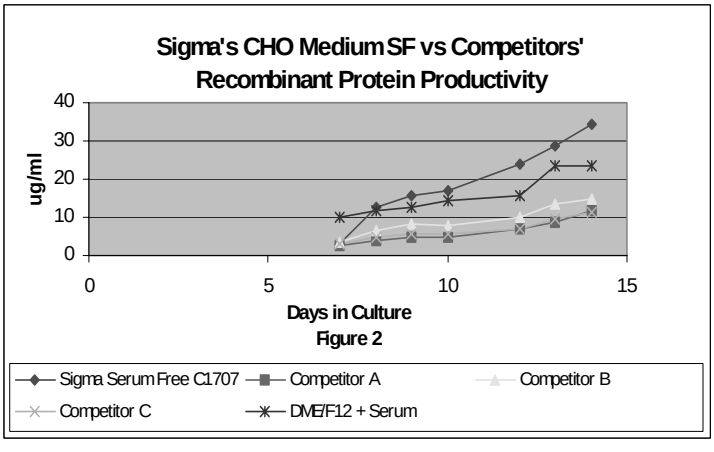

Figure 2